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PERMANENT GENETIC RESOURCES

Microsatellites from kousa dogwood (*Cornus kousa*)

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Abstract

Microsatellite loci were identified from *Cornus kousa* 'National'. Primer pairs for 86 loci were developed and of these, eight were optimized and screened using genomic DNA from 22 kousa cultivars. All optimized loci were polymorphic and the number of alleles per locus ranged from three to 17. Observed heterozygosity ranged from 0 to 0.3 and expected heterozygosity ranged from 0.38 to 0.91. These microsatellites will be useful in population studies, and a breeding programme for cultivar development of *Cornus* species.

Keywords: genomic library, PCR, SSR enrichment

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Cornus kousa Hance is considered by Cappiello & Shadow (2005) to be the Asian ornamental counterpart of the North American flowering dogwood (*Cornus florida* L.) and of the over 55 species in the genus is most closely related to *C. florida* in the big-bracted group as described by Fan & Xiang (2001). There are over 100 cultivars of kousa dogwood and as with flowering dogwood, the showy bracts are the primary selected orna-mental trait, which range from white to cream and from red to pink. Kousa dogwood has increased in popularity because it is more resistant to diseases than flowering dogwood (Ranney *et al*. 1995; Mmbaga & Sauvé 2004). Microsatellites were recently developed for flowering dogwood (Cabe & Liles 2002; Wang *et al*. 2007). Here, we report the development of microsatellites from *C. kousa* 'National' using a biotin enrichment protocol and test their cross amplification in 22 kousa cultivars. These microsatellites will be useful in assessing the genetic diversity of the species and diversity among *C. kousa* cultivars that are sold as ornamental trees.

Genomic libraries enriched for $(CT)_n$- and $(GT)_n$-containing sequences were constructed based on Wang *et al*. (2007). DNA (5–25 μg) was digested with *Alu*I, *Hae*III and *Rsa*I and ligated to SNX linker adaptors (Hamilton *et al*. 1999) and then polymerase chain reaction (PCR) amplified using SNX linker primers. The PCR products were column purified with a QIAquick PCR Purification Kit (QIAGEN) and hybridized to $(CT)_{12}$ and $(GT)_{12}$ biotinylated oligonucleotides to select for fragments containing $(CT)_n$ or $(GT)_n$ microsatellite sequences. The fragments were bound using MagneSphere streptavidin-coated paramagnetic particles (Promega), eluted and PCR amplified with the SNX linker primer. The enriched fragments were purified with the QIAquick PCR Purification Kit (QIAGEN). Purified PCR products were ligated to *Eco*R V-cut pBluescript SK II (+) DNA and then transformed into electrocompetent *Escherichia coli* TG-1 cells (Stratagene). Transformed cells were plated onto Luria-Bertani-Amp_{100} with IPTG and X-Gal and white colonies were transferred into 96-well plates containing Luria-Bertani freezing medium (Sambrook *et al*. 1989) with 100 μg/mL of ampicillin. Individual colonies were incubated overnight and colony PCR was used to screen for microsatellites using 10 μL PCRs consisting of the following: 1× GeneAmp PCR buffer II (Applied Biosystems), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM T3 primer, 0.5 μM T7 primer, 0.5 μM $(CT)_n$ or $(GT)_n$ oligo, 0.2 U Ampli*Taq* Gold DNA Polymerase (Applied Biosystems) and water. The PCR was amplified using the following conditions: 1 cycle at 95 °C for 3 min, 35 cycles at 95 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and 1 cycle at 72 °C for 1 min. PCR products were separated on 2% agarose gels and colonies considered positive for an inserted microsatellite motif if a smear was present in the gel (Wang *et al*. 2007). Plasmid DNA of positive colonies was isolated using a modified

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Table 1 Microsatellite loci in *Cornus kousa*. Locus designations are given along with, repeat motifs, forward (F) and reverse (R) primer sequence (5′–3′ direction) as well as allele size range. Allele number (*A*), observed (H_O) and expected (H_E) heterozygosities were calculated for all 22 kousa dogwood cultivars

Locus*	Primer sequences (5′–3′)	Repeat	Allele size range (bp)	*A*	H_E	H_O
CK007	F: GAGCCCAGAAGAAGAATATAGAC	$(AG)_8$	109–111	3	0.45	0.00†
	R: ATATAATTGGGTTGGGTTTTG					
CK015	F: GTCAAATTTTTGATCTTTCTCTCT	$(CT)_{10}$	104–136	17	0.91	0.24†
	R: GGAGAGACAGAGTACAGTAGAGGT					
CK029	F: AATTTAGGTTAAGGTTTTGATTTG	$(TC)_8$	101–108	7	0.65	0.24†
	R: AGAGAGAATAGGTTACAGCATCAT					
CK031	F: TGTCACTGCTTACAGAAACAAT	$(CT)_7$	114–138	7	0.76	0.29†
	R: TATGACGAGATTGTATAAGTTGCT					
CK040	F: CCAAGTCAGTTTGGTAGTAATTC	$(GT)_{16}$	80–114	10	0.88	0.20†
	R: AGTGCAACTTTTACTTGCTATGT					
CK043	F: TTGAGACCCTCTTCATAGTCTAGT	$(AT)_6(GT)_9$	112–126	4	0.38	0.15†
	R: CTACAATCCTAAACAGCTAAACAA					
CK047	F: GAAAGAGATAAAAGATGGTTCAAT	$(AT)_4(AC)_6$	128–134	6	0.79	0.30†
	R: CTTATAGAGTAAGCCCACCATC					
CK048	F: ACCAACCAAAAGAAGTATAAAGAA	$(TA)_6$	151–179	11	0.80	0.21†
	R: CCTATAAATAAGGAGTGATTTGGT					

*GenBank Accession nos EU125522–EU125529; †significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

alkaline lysis method and sequenced (ABI Big-Dye version 3.1 terminators) on a Model ABI 3730XL capillary electrophoresis DNA sequencer, assembled using CAP 3 (Huang & Madan 1999) and microsatellites identified with SSR FINDER (MMP software). Primers were designed using PRIMER 3.0 (Whitehead Institute of Biochemical Research) and synthesized by Integrated DNA Technologies.

Overall, 416 of 576 (72%) of sequenced colonies contained a microsatellite with the desired motifs and 86 primer pairs were designed. Eight primer pairs were optimized to amplify loci of 22 kousa cultivars (Autumn Rose, Beni Fuji, Big Apple, Blue Shadow, Bodnant, Bush's Pink, Doubloon, Elizabeth Lustgarten, Emerald Star, Galilean, Greensleeves, Milky Way, Milky Way Select, Miss Satomi, National, Rochester, Snow Flake, Spinners, Steeple, Summer Majesty, Temple Jewel, Trinity Star). Microsatellite amplification was completed using the following conditions: 10 μL PCR containing 0.4 ng genomic DNA, 2.5 mM $MgCl_2$, 1× GeneAmp PCR buffer II (Applied Biosystems), 0.2 mM dNTPs, 0.25 μM primer, 0.2 U Ampli*Taq* Gold DNA polymerase (Applied Biosystems), and water. Cycling conditions were 1 cycle of 94 °C for 5 min, 35 cycles of 94 °C for 40 s, 58 °C for 40 s, 72 °C for 30 s, and 1 cycle of 72 °C for 4 min. PCR products were sized on the HAD-GT12 Capillary Electrophoresis System (eGene) using an internal 25–300 bp size standard. All loci were polymorphic and produced clear and reproducible electropherograms (Table 1).

Allele number per locus ranged from three to 17. Observed heterozygosity ranged from 0 to 0.3 and expected heterozygosity ranged from 0.38 to 0.91. Tests of Hardy–Weinberg equilibrium and linkage disequilibrium between all pairs of loci were conducted using GENEPOP (Raymond & Rousset 1995). All loci showed significant deviations from Hardy–Weinberg expectations. These deviations probably resulted from a small population size and nonrandom mating and selection of cultivars with desirable traits from existing germplasm. None of the pairwise comparisons among loci exhibited linkage disequilibrium. The 22 cultivars used in this study represent a broad spectrum of ornamental characters and disease resistance found in kousa dogwood. This diversity is reflected in the polymorphic content of some loci, particularly CK015, CK040, CK048, which had the most alleles.

We expect to use these loci for population genetic studies as well as identification of kousa dogwood cultivars. We are testing cross amplification of these loci in other *Cornus* species in an effort to verify intraspecific and interspecific hybrids, which are important to future dogwood cultivar breeding. Optimization of simple sequence repeat amplification was relatively straightforward and we expect the remaining 78 simple sequence repeat loci to be useful in future studies.

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